that traverses these gap junctions during instructive left-right patterning. Based on existing data, strong candidates include electrical currents, Ca^{2+}, and serotonin (which is thought to be the morphogen that moves through gap junctions during establishment of frog asymmetry; Levin, 2006). The relationship of this pathway to other known components that establish *C. elegans* asymmetry (such as *Notch* signaling and the ASE system) also remains to be worked out. Most crucially, are other innexins involved? With the discovery that even a species in which cell lineages are highly determined uses gap junctions as part of its repertoire for generating left-right asymmetry, it is clear that additional surprises await us in the exploration of physiological intercellular signals in the establishment of laterality.

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Inositol Pyrophosphates Get the Vip1 Treatment

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Inositol pyrophosphates are unique signaling molecules implicated in the regulation of diverse cellular processes. Two new studies by Mulugu et al. (2007) and Lee et al. (2007) extend the biological and metabolic diversity of this class of molecules. They identify yeast Vip1 as a new inositol pyrophosphate synthase and show that the products of Vip1 activity regulate a cyclin/cyclin-dependent kinase complex.

The fully phosphorylated six-carbon ring of IP_6 (phytic acid) was thought to represent the end point of inositol phosphorylation. The discovery of inositol phosphate species with seven (diphosphoinositol pentakisphosphate; PP-IP_5; IP_7) or eight phosphates (bis-diphosphoinositol tetrakisphosphate; $[PP]_2$-IP_4; IP_8) on the inositol ring was exciting and unexpected (Menniti et al., 1993; Stephens et al., 1993). These "high-energy" molecules have been linked to a wide range of biological functions, including vesicle trafficking, apoptosis, DNA repair, telomere maintenance, and the stress response (Bennett et al., 2006). Although the mechanisms of action of inositol pyrophosphates in these cellular processes remain unclear, IP_7 has been shown to directly transfer the energetic β phosphate of the pyrophosphate moiety to multiple proteins, indicating that IP_7 may regulate signaling pathways through a new mechanism of protein phosphorylation (Saiardi et al., 2004). Two new studies published in *Science* identify an enzyme that catalyzes the production of inositol pyrophosphates (Mulugu et al., 2007) and a new target of regulation by IP_7, a cyclin/cyclin-dependent kinase complex (Lee et al., 2007).

The inositol hexakisphosphate kinases (IP6K) are the enzymes responsible for synthesis of inositol pyrophosphates (Saiardi et al., 1999). They are highly conserved evolutionarily and at least one member of this enzyme family is present in all eukaryotic genomes sequenced so far (Bennett et al., 2006). Inositol pyrophosphates and their kinases have been predominantly

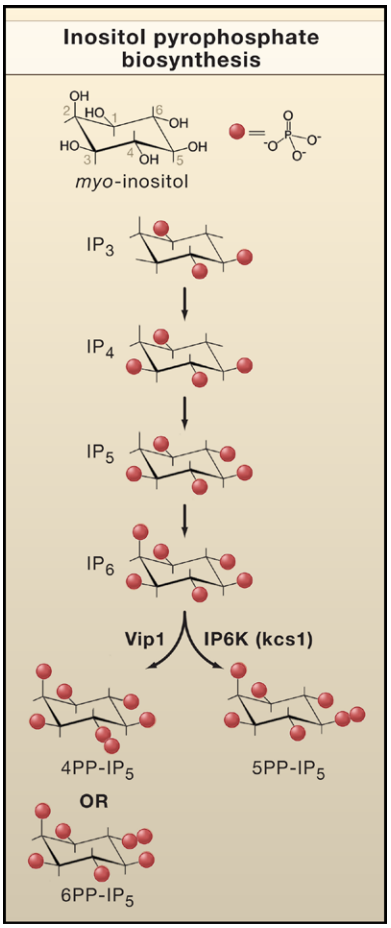

Figure 1. The Biosynthetic Pathway of Inositol Pyrophosphates

Myo-inositol (shown with six hydroxyl groups: five equatorial and one axial at position C2) is the building block of inositol polyphosphates. Sequential kinase reactions convert IP_3 to IP_4, IP_5, and IP_6. The fully phosphorylated ring is then converted to IP_7 either by the action of the inositol pyrophosphate synthase Kcs1, generating the isomer 5PP-IP_4, or by the newly identified enzyme Vip1, generating 4/6PP-IP_4.

studied in the budding yeast *Saccharomyces cerevisiae*, particularly using mutants lacking the single yeast IP6K, Kcs1. In the *kcs1* deletion mutant, the naturally occurring inositol pyrophosphates IP_7 and IP_8 are virtually undetectable. Surprisingly, an additional deletion of the IP_7 phosphatase DDP1 in this mutant resulted in the reappearance of IP_7, suggesting the existence of a second enzyme that can synthesize IP_7 (York et al., 2005).

Using cell extracts prepared from yeast lacking both Kcs1 and DDP1, Mulugu et al. (2007) biochemically purified a second inositol pyrophosphate synthase, which they call Vip1. Protein sequence analysis revealed two conserved domains in Vip1: an amino-terminal ATP-grasp superfamily domain with IP6 kinase activity and a histidine acid-phosphatase domain in the C terminus (Mulugu et al., 2007). The generation of yeast lacking Kcs1, DDP1, and Vip1 resulted in ablation of IP_7, which could be restored by reintroducing Vip1, confirming that Vip1 produces IP_7 in vivo (Mulugu et al., 2007). The most important finding of the Mulugu et al. work is that Vip1 synthesizes a form of IP_7 that is distinct from the isomers 5PP-IP_5 produced by human IP6K1 (which may have similar activity to yeast Kcs1). The IP_7 generated by Vip1 has the pyrophosphate moiety located at either the C4 or C6 position (4/6PP-IP_5) producing enantiomers that cannot be distinguished (Figure 1). Vip1 and Kcs1 together produce IP_8, but it is unclear if Vip1 is equivalent to the previously isolated IP_7-kinase activity (Huang et al., 1998).

Insight into the physiological role of Vip1 comes from studies of Asp1, its ortholog in fission yeast *Schizosaccharomyces pombe*, which regulates actin-related protein (Arp) complexes. Functional analyses revealed that IP6 kinase activity is essential for maintaining cellular integrity, normal growth, and genetic interactions with Arp complex components.

Mulugu and colleagues indisputably demonstrate that Vip1 is required to produce the IP_7 present in yeast lacking both Kcs1 and DDP1. Given that yeast lacking Kcs1 have nearly undetectable levels of inositol pyrophosphates, how much of the total IP_7 in cells is synthesized by Vip1? This might be determined by analyzing the single mutant lacking Vip1 and double mutants deficient in both Vip1 and DDP1. The most interesting characteristic of Vip1 is its dual domain architecture, although the specificity of the phosphatase domain has yet to be determined.

In a companion study, Lee et al. (2007) elegantly demonstrate that 4/6PP-IP_5, the form of IP_7 generated by Vip1, but not 5PP-IP_5, the form generated by Kcs1, is a regulator of the Pho80-Pho85 cyclin/cyclin-dependent kinase (CDK) complex. This CDK complex is a key component of the PHO phosphate response pathway in yeast. Using a biochemical approach, Lee et al. (2007) sought to identify cellular components that control the phosphate starvation response initiated when cells are grown in media low in phosphate. In *S. cerevisiae*, the Pho80-Pho85 cyclin-CDK complex is a key regulator of the PHO signaling pathway. Under high-phosphate conditions, the Pho80-Pho85 complex is active and phosphorylates the transcription factor Pho4, which is then exported from the nucleus into the cytoplasm. In contrast, when cells are starved of phosphate, the Pho80-Pho85 complex is inhibited, leading to nuclear accumulation of dephosphorylated Pho4 and transcription of PHO genes. The Pho81 CDK inhibitor is constitutively bound to the Pho80-Pho85 complex and is required for its inhibition in response to phosphate limitation. Analysis of fractionated extracts from phosphate-starved cells led Lee et al. to identify a small molecule capable of inhibiting the Pho80-Pho85 kinase activity in a Pho81-dependent manner. This molecule is IP_7. Importantly, inactivation of Pho80-Pho85 by IP_7 is specific because inositol and many of its phosphorylated derivatives did not affect kinase activity (Lee et al., 2007). That Vip1 is the IP6 kinase involved in controlling the PHO pathway was shown by assaying extracts from phosphate-starved cells lacking Kcs1 and Vip1 for their ability to inactivate Pho80-Pho85-Pho81. Only extracts from cells lacking Kcs1 but containing Vip1 were able to inhibit Pho80-Pho85 in a Pho81-dependent manner. Furthermore, the IP_7 (4/6PP-IP_5) synthesized by Vip1 inhibits the Pho80-Pho85 complex, whereas the 5PP-IP_5 isomer synthesized by human IP6K1 does not (Lee et al., 2007). Interestingly, IP_7 levels increased in response to phosphate limitation, leading the authors to conclude that when cells are starved of phosphate, the IP_7 concentration increases and thus inhibits the Pho80-Pho85-Pho81 complex. However, in our laboratory, using a different yeast strain and slightly different experimental condi-

tions, we observed a decrease in IP_7 cellular concentrations in response to phosphate starvation (A.S., unpublished data), suggesting that more characterization of this response may be necessary.

Lee et al. convincingly provide a link between Vip1-mediated inositol pyrophosphate signaling and phosphate metabolism. However, previous reports have also linked Kcs1 and its IP6K family members to phosphate metabolism, suggesting that both 5PP-IP_5 and 4/6PP-IP_5 have roles in these processes. Indeed, the first clue to a relationship between inositol pyrophosphate signaling and phosphate metabolism came from the discovery that PiUS, a stimulator of inorganic phosphate uptake, was in fact IP6K2 (Bennett et al., 2006). Then it was shown that inorganic phosphate uptake in yeast lacking Kcs1 is less efficient than in wild-type cells. Yeast lacking Kcs1 also have increased expression of the PHO phosphate response genes and reduced intracellular polyphosphate levels (Auesukaree et al., 2005; Bennett et al., 2006). Moreover, yeast lacking Kcs1 constitutively express the acid phosphatase PHO5, which is activated by Pho4, indicating that the IP_7 synthesized by Kcs1 also influences the PHO transcriptional pathway (Auesukaree et al., 2005). These observations may reflect different aspects of IP_7 function in phosphate sensing. It is possible that 4/6PP-IP_5 mainly regulates the PHO pathway by protein binding, whereas 5PP-IP_5 primarily functions as a phosphate donor.

The two new studies raise several exciting possibilities that could advance our understanding of inositol pyrophosphates and their roles in many different aspects of cell signaling. Certainly, the notion that two structurally different IP_7 molecules can have distinct cellular roles is intriguing and will no doubt guide the way to more pioneering work. In the past decade, a large number of inositol pyrophosphate species, not only IP_7 and IP_8, have been discovered as well as several inositol pyrophosphate synthases and phosphatases. Further studies into these fascinating signaling molecules may result in the discovery of other species, perhaps even pyrophosphorylated inositol lipids.

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Guiding Ligands to Nuclear Receptors

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Retinoic acid—the active metabolite of vitamin A—influences biological processes by activating the retinoic acid receptor (RAR). In this issue, Schug et al. (2007) demonstrate that retinoic acid also activates the peroxisome proliferator-activated receptor β/δ (PPARβ/δ). Remarkably, retinoic acid signaling through RAR or PPARβ/δ—which depends on cytoplasmic retinoic acid transporters—commits the cell to opposite fates, apoptosis or survival, respectively.

The beneficial effects of vitamin A might have been recognized first by the ancient Egyptians, who treated eye disease with raw liver. The fact that they recognized a connection between eye problems and the liver, which is the richest source of dietary vitamin A, is astonishing. Today, we know that vitamin A (retinol) and its biologically active derivatives, the retinoids (the most potent of which is all-*trans*-retinoic acid), regulate key processes such as inhibition of cell proliferation, differentiation, apoptosis, shaping of the embryo,